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(54) Title: NERVE REGENERATION .

(57) Abstract: A nerve guide for use in promoting the *in vivo* regeneration and/or extension of a nerve, comprises at least one tubular conduit, the at least one conduit having an opening at a proximal end to receive at least one neurite, and an inner lumen through which the neurite may regenerate and/or extend. The inner lumen has an internal diameter which is less than the diameter of the nerve to be regenerated. A method for regenerating severed or otherwise damaged nerves is also described.

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## NERVE REGENERATION

### INTRODUCTION

The invention relates to the regeneration of nerves, especially severed, crushed or otherwise damaged nerves. The invention also relates to a method of regenerating such damaged nerves.

Many different methods have been proposed for the regeneration of a nerve that has been subjected to trauma. One such technique involves suturing the proximal and distal ends of a severed nerve. However, scar tissue resulting from the surgery has been found to interfere with nerve growth at proximal nerve ends. Microsurgical nerve grafting has also been used to treat severe nerve injuries. This technique, which involves physically grafting a piece of nerve from another part of the body into a gap in the damaged nerve, has met with limited success due to the inherent problems with removing substantial amount of nerve tissue for grafts. Moreover, both of the above techniques may result in neuroma.

Various attempts have been made to find a replacement for direct, i.e., nerve to nerve, suturing or grafting. Much of the research has focussed on the use of channels or tubular prostheses which allow the cut ends of a nerve to be secured in relative proximity without undue trauma (see for example US3960151, US4662884, US4877029, US4963146, US5019087 and US5834029). All of these documents generally disclose the use of a bridging conduit which has a bore of sufficient diameter to receive the ends of a severed nerve as illustrated in Fig 1a. In use, the end of the cut nerve may regenerate and extend through the conduit towards an opposite end of the nerve. In practice this technique has only met with limited success. Moreover it is limited by the distance along which nerves will regenerate and the speed of regeneration.

It is an object of the invention to overcome at least some of the above problems.

## STATEMENTS OF INVENTION

The present invention is based on the surprising discovery that microconduits having a diameter smaller than the diameter of the nerve to be regenerated not only direct nerve regeneration, but significantly accelerate neurite extension. Accordingly, the present invention broadly relates to a nerve guide for use in promoting the in-vivo regeneration and/or extension of a nerve, comprising at least one tubular conduit, the at least one conduit having an opening at a proximal end to receive at least one neurite, and an inner lumen through which the neurite may regenerate and/or extend, wherein the inner lumen has an internal diameter which is less than the diameter of the nerve to be regenerated.

Typically, the inner lumen has an internal diameter of between 1 and 1000 microns, preferably less than 900 microns, more preferably less than 800 microns, more preferably less than 700 microns, more preferably less than 600 microns, more preferably less than 500 microns.

In one embodiment of the invention, the nerve guide comprises a plurality of tubular conduits. Typically, the conduits will be of equal length and equal internal diameter. Preferably, the conduits will be bundled together and ideally fixed in a bundled arrangement by a suitable fixing means such as an adhesive. Thus the nerve guide may comprise a bundle of individual conduits, each conduit having an internal diameter which is less than the diameter of the nerve to be regenerated. Generally, when intended for use with nerves having a diameter greater than 1mm, the conduits will have a diameter which is considerably less than 1mm. Thus, in one embodiment of the invention, the conduit will have a diameter which is less than 50%, preferably less than 40%, more preferably less than 30%, more preferably less than 20%, and most preferably less than 10% of the diameter of the nerve. Typically, the guide will comprise between 1 and 100, preferably between 1 and 50, more preferably between 1 and 25, more preferably between 1 and 10, conduits.

In one particularly preferable embodiment of the invention the bundle of conduits has a diameter which is approximately equivalent to the diameter of the nerve.

Suitably, the lumen of the or each conduit is filled with a hydrogel. Hydrogels suitable for use with the nerve guides on the invention will be known to those skilled in the art of nerve tissue engineering. Particularly suitable hydrogel compositions are those based on collagen, fibrin or agarose.

Preferably, the hydrogel contains molecules that stimulate neurite extension, such as for example laminin, heparin, heparin sulphate proteoglycan, glycosaminoglycans (such as chondroitin sulphate and hyaluronic acid), growth hormones such as epidermal growth factor (EGF), nerve growth factor, glycoproteins such as fibronectin and the like.

In one embodiment of the invention, the or each conduit comprises walls which are impermeable to cells and macromolecules such as proteins, peptides and polysaccharides.

Preferably, the walls are impermeable to micromolecules such as amino acids, carbohydrates and the like. Ideally, the walls are gas impermeable. Suitably, the conduits are formed of glass. Most of the prior art documents disclose the use of conduits having walls which are permeable to the diffusion of certain desirable constituents which feed and aid the nerve regeneration within the conduit. Without being bound by theory, the Applicant believe that conduits having substantially impermeable walls may promote growth of the neurites within the conduit by denying them nutrients, whereby the neurites are encouraged to grow along the conduit towards a distal end. In this regard, growth factors may be disposed at or adjacent a distal opening of the or each conduit.

In one embodiment of the invention, the nerve guide further includes an external sheath which is dimensioned to embrace the conduit, or the bundle of conduits, and the ends of the nerve. The sheath may be formed of a material which is substantially impermeable macromolecules, preferably substantially impermeable to micromolecules, and most preferably gas impermeable.

The invention also relates to a nerve guide for use in regenerating severed or otherwise damaged nerves, comprising a conduit having an opening at each end which communicate through an inner lumen, wherein the walls of the conduit are impermeable to macromolecules. Preferably, the walls are impermeable to micromolecules. More preferably the walls are gas impermeable.

The invention also relates to a nerve guide for use in regenerating severed or otherwise damaged nerves, comprising a conduit having an opening at each end which communicate through an inner lumen, wherein the inner lumen has an internal diameter between 1 and 650 microns, preferably between 1 and 500 microns, preferably between 1 and 400 microns, preferably between 1 and 300 microns, preferably between 1 and 200 microns. Suitably, the internal diameter is less than 100 microns.

The invention also relates to a use of a nerve guide according to the invention for promoting the regeneration or extension of a nerve having a diameter which is greater than the internal diameter of a conduit in the nerve guide.

The invention also relates to a method for promoting the *in vivo* extension of a nerve towards a target site comprising the steps of: providing a nerve guide according to the invention; positioning a proximal end of the guide adjacent an end of the nerve; and positioning a distal end of the conduit adjacent the target site, wherein the target site typically comprises an endoneural sheath.

## DETAILED DESCRIPTION OF THE INVENTION

The invention will be more clearly understood from the following description of some embodiments thereof given by way of example only with reference to Figs 1b and 1c. Referring initially to Fig 1b, a nerve guide according to the invention, indicated generally by the reference numeral 1, comprises a plurality of conduits 2 fixed together in bundle. Each conduit 2 comprises a tubular channel having an internal diameter of 250 microns, and an opening at a proximal end 4 and a distal end 5, which openings

communicate via an inner lumen (not shown). As can be seen, each conduit 2 has an internal diameter which is significantly less than the diameter of the nerve 6, and the diameter of the guide 1 is approximately the same size as the diameter of the nerve 6. In use, the guide 1 is used to bridge a gap between the ends 7a, 7b of the severed nerve 6, and is positioned such that the proximal end of each conduit lies adjacent a distal cut end of the nerve 7a, and the distal end of each conduit lies adjacent a proximal end of the cut nerve 7b. In this orientation, neurites from the nerve 6 will grow into and along the conduits 2 towards a target site, which in this case is the opposite end of the severed nerve.

Referring to Fig 1c, an alternative embodiment of the nerve guide is illustrated in which parts similar to that described with reference to the previous embodiment are given the same reference numerals. In this embodiment, the nerve guide further includes an external sheath 10 which embraces the ends 7a, 7b of the severed nerve 6 and the bundle of conduits 2.

#### **Brief Description of Drawings**

**Figure 1:** a, b, c, schematic.

**Figure 2:** Elongation of neurites propagated from excised Wistar rat dorsal root ganglia. Explants positioned on type I collagen gel at one end of an open-ended glass conduit, with internal diameter of 635 $\mu$ m. Maximum length of intra-conduit neurite propagation ( $\rightarrow\blacklozenge\rightarrow$ ), Maximum length of extra conduit neurites ( $\rightarrow\blacksquare\rightarrow$ ).

**Figure 3:** Points indicate individual values for neurites propagated from Wistar DRG cultures 20 days post explant.

**Figure 4:** Neurites propagated from Wistar rat dorsal root ganglia through a 635 $\mu$ m collagen Type I filled micro-conduit, following 19 days in culture (37°C/5%CO<sub>2</sub>).

**Figure 5:** Neurites propagated from a Wistar rat dorsal root ganglia explant. Neurites are growing both within and outside a 285 $\mu$ m type I collagen filled micro-conduit, following 11 days in culture (37°C/5%CO<sub>2</sub>).

## **EXPERIMENTAL**

### **Dorsal Root Ganglia Isolation**

Cervical and lumbar dorsal root ganglia (DRG) were excised, with the aid of a dissection microscope, from 180g Wistar strain *Mus norvegicus albinus*. DRG were collected in Dulbecco's Modified Eagles Medium (DMEM) and de-sheathed with the tines of number 5 Dumont Biology forceps. De-sheathed DRG were incubated in collagenase dissolved in phosphate buffered saline (PBS), at 37°C for approximately 20 minutes. DRG were then rinsed twice in PBS.

### **Glass Conduits**

Glass conduits were prepared from disposable glass micro-pipettes. 3mm lengths of glass conduit were prepared by scoring and snapping. The internal diameters of the conduits were: 200, 280, 400 and 600 microns.

### **Tissue Culture Medium**

DRG explants were cultured in medium prepared from a 50:50 mixture of Ham's F12 and DMEM, containing insulin, transferrin, putrescine, progesterone, sodium selenite, 2.5S nerve growth factor (NGF), penicillin G, streptomycin sulphate and amphotericin B.

### **Collagen Lattices**

A 0.3% solution (pH3) of acid solubilized type I collagen was used to prepare collagen lattices. The lattices were composed of Hank's balanced salt solution (HBSS), N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid (HEPES), sodium bicarbonate, collagen type I, and deionised water. All reagents were cooled to 4°C and mixed and kept on ice until required.

### **Experimental Procedure**

Collagen lattice solution was placed in polystyrene culture vessels and gelled by placing in an incubator (37°C/5%CO<sub>2</sub>). 3mm lengths of glass conduits were filled with collagen lattice solution (4°C), either using capillary

action alone or with assistance of a diaphragm vacuum pump. Conduits containing collagen lattice solution were placed on the surface of collagen lattice coated polystyrene culture vessels, the intra-conduit collagen lattice solution was gelled by incubating at 37°C/5%CO<sub>2</sub>.

DRG explants, prepared as described above, were precisely placed at one end of the glass conduit. The polystyrene culture vessels containing the collagen lattice, glass conduits and DRG explants were immersed in Ham's F12-DMEM medium, prepared as described above.

The propagation and elongation of neurites from DRG explants was monitored using an inverted microscope and Köhler illumination (Leica DMIRB). Images were acquired using a JVC TK-C1380 colour video camera, Matrox Meteor Frame grabber, and Leica Qwin image analysis software. Image analysis was performed on composite images constructed from several micrographs in the form of a collage.

### Experimental Results

*In vitro* explant cultures of Wistar rat dorsal root ganglia (DRG) have shown that micro-conduits increase neurite extension above levels observed extra-conduit. Firstly, the rate of neurite extension is increased, markedly during the 0-6 day culture period (Figure 2). Secondly, the maximum lengths of the intra-conduit neurites are markedly longer than extra-conduit neurites between the 4-20 day period investigated (Figure 3). Neurites growing inside a 635µm conduit are depicted in Figure 4. Thirdly, similar results were obtained throughout a range of micro-conduit channel diameters (285-635µm). Figure 5 shows neurites propagated from a DRG explant both outside and within a 285µm conduit.

The invention is not limited to the embodiments hereinbefore described which may be varied in both construction and detail without departing from the spirit of the invention.

**CLAIMS**

1. A nerve guide for use in promoting the in-vivo regeneration and/or extension of a nerve, comprising at least one tubular conduit of a biocompatible material, the at least one conduit having an opening at a proximal end to receive at least one neurite, and an inner lumen through which the neurite may regenerate and/or extend, wherein the inner lumen has an internal diameter which is less than the diameter of the nerve to be regenerated.
2. A nerve guide as claimed in claim 1, in which the inner lumen has an internal diameter of from 1 to 1000 microns.
3. A nerve guide as claimed in claim 2, in which the conduit has an internal diameter of from 100 to 700 microns.
4. A nerve guide as claimed in any one of claims 1 to 3, having a plurality of tubular conduits.
5. A nerve guide as claimed in claim 4, in which the tubular conduits are bundled together.
6. A nerve guide as claimed in claim 5, in which the bundle of conduits has a diameter which is approximately equivalent to the diameter of the nerve.
7. A nerve guide as claimed in any one of claims 1 to 6, in which the diameter of each conduit is substantially less than the diameter of the nerve to be regenerated, typically less than one third of the diameter of the nerve to be regenerated.

8. A nerve guide as claimed in any one of claims 1 to 7, in which the lumen of the or each conduit is filled with a hydrogel.
9. A nerve guide as claimed in claim 8, in which the hydrogel comprises one or more of collagen, fibrin and agarose.
10. A nerve guide as claimed in either claim 8 or claim 9, in which the hydrogel contains molecules that stimulate neurite extension.
11. A nerve guide as claimed in any one of claims 1 to 10, in which the or each conduit comprises walls which are impermeable to cells and macromolecules.
12. A nerve guide as claimed in claim 11, in which the walls are impermeable to micromolecules.
13. A nerve guide as claimed in claim 12, in which the walls are gas impermeable.
14. A nerve guide as claimed in any one of claims 1 to 13, in which the conduits are formed of glass.
15. A nerve guide as claimed in any one of claims 1 to 14, in which growth factors are disposed at or adjacent a distal opening of the or each conduit.
16. Use of the nerve guide claimed in any one of claims 1 to 15 for promoting the regeneration or extension of a nerve having a diameter which is greater than the internal diameter of a conduit in the nerve guide.

17. A method for promoting the in-vivo extension of a nerve towards a target site comprising the steps of:
  - a. providing a nerve guide according to any one of claims 1 to 15;
  - b. positioning a proximal end of the guide adjacent an end of the nerve; and
  - c. positioning a distal end of the conduit adjacent the target site.
18. A method as claimed in claim 17, in which the target site comprises an endoneural sheath.

FIGURE 1

Fig. 1a

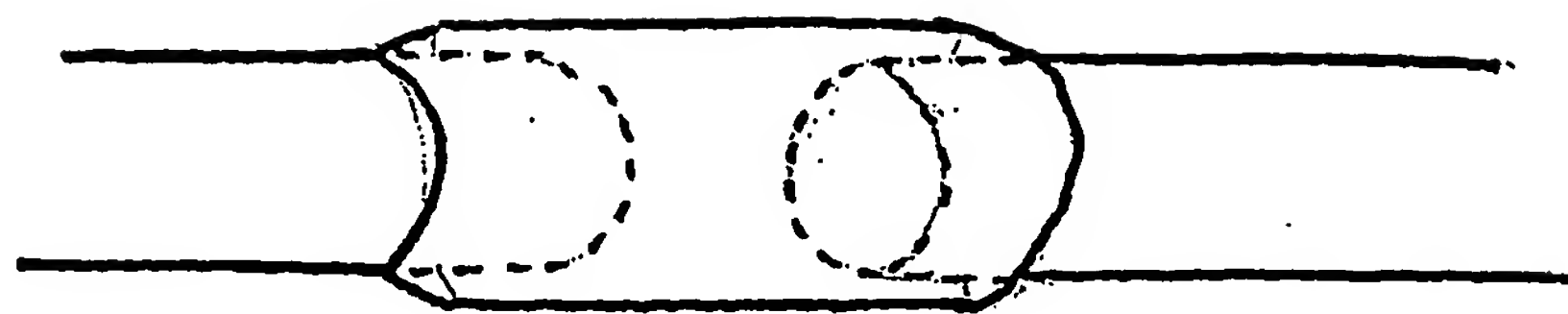


FIG 1b

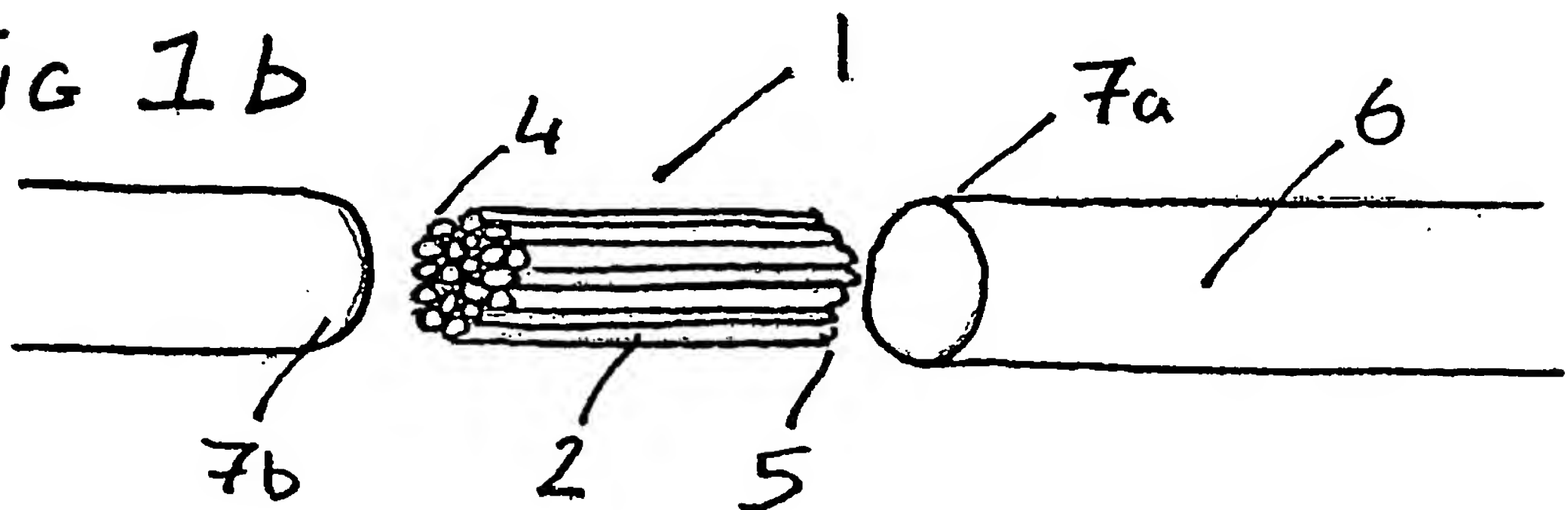


FIG. 1c

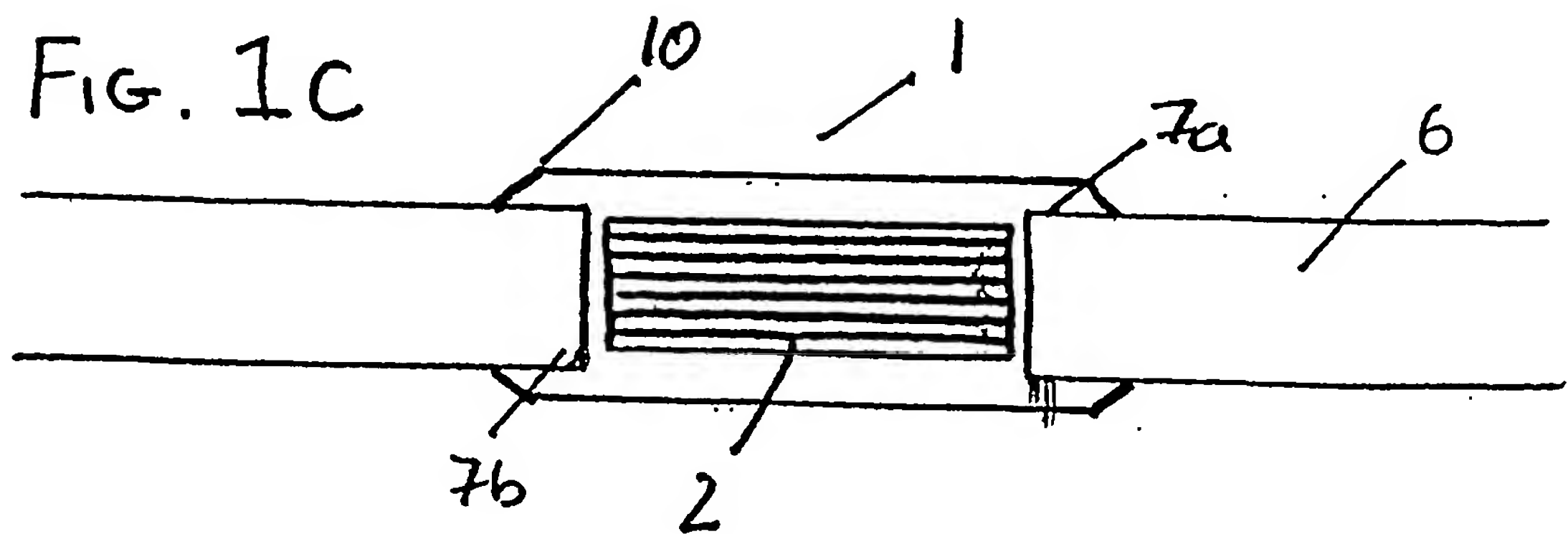
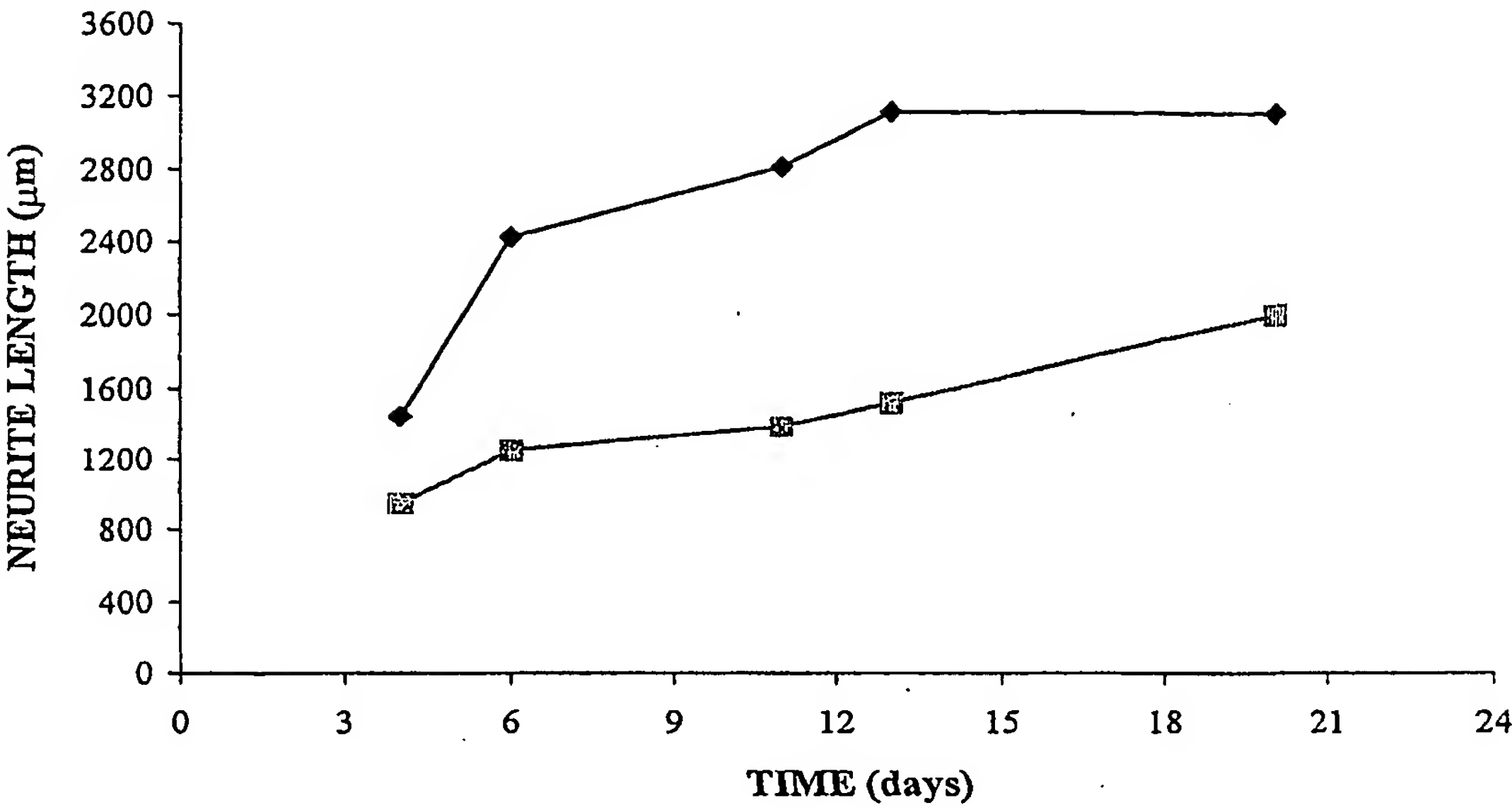


FIGURE 2



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FIGURE 3

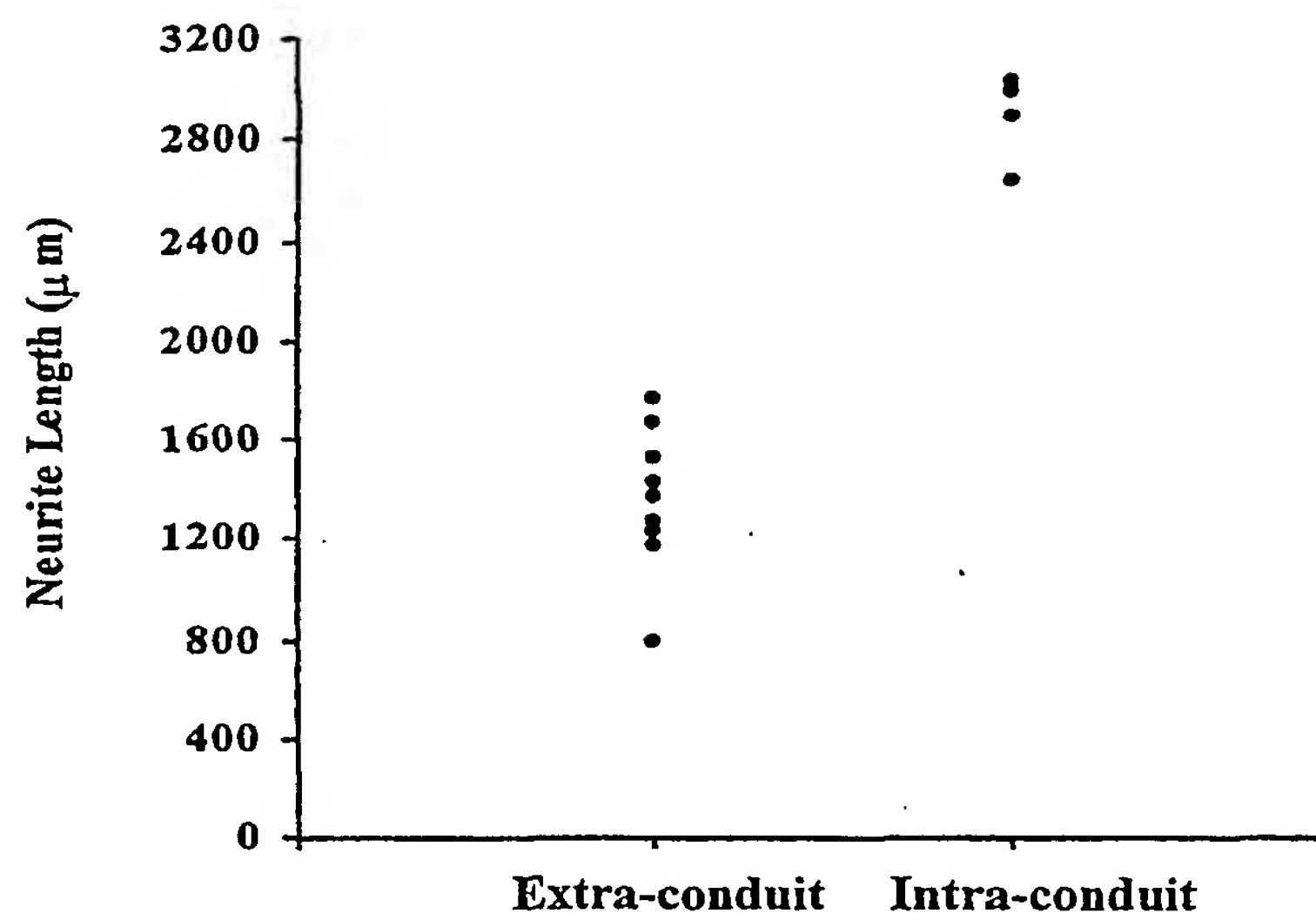


FIGURE 4

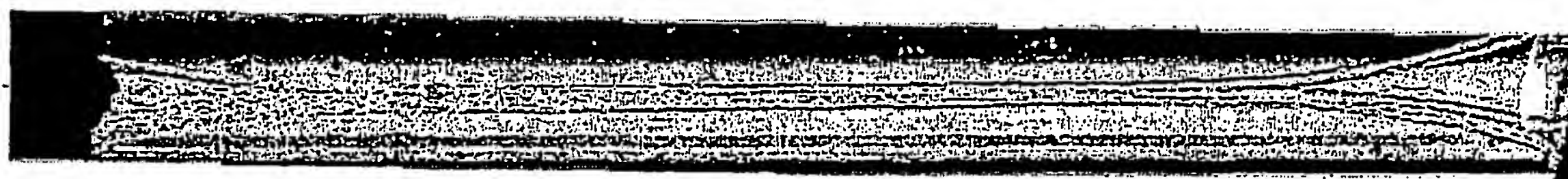
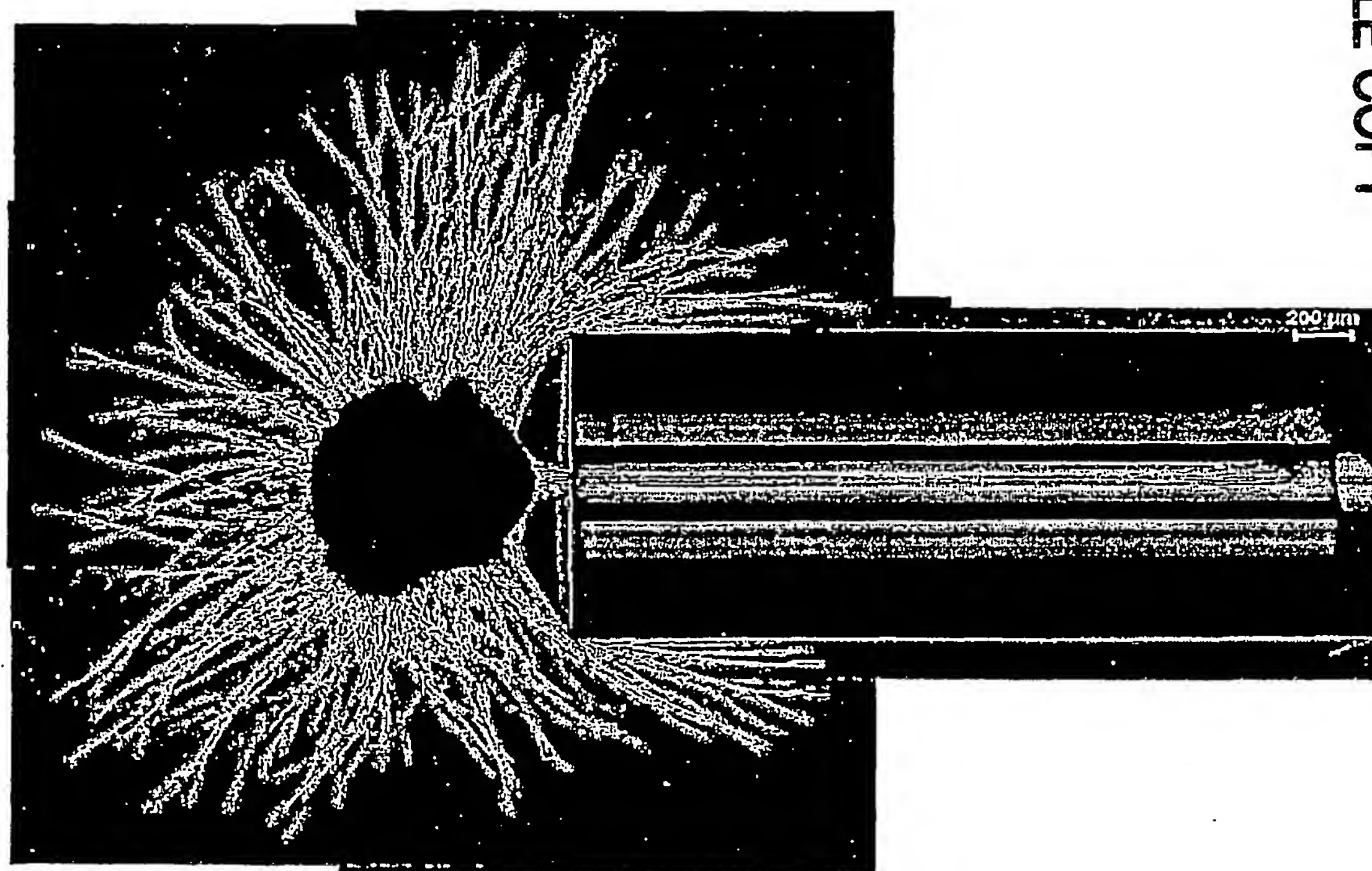


FIGURE 5



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A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61B17/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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